MOTILITY AND POWER DISSIPATION IN FLAGELLATED CELLS, ESPECIALLY CHLAMYDOMONAS[1]

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The energetics of cellular motion have evoked much interest over the past few decades. Muscle, amoeboid cells, and ciliated or flagellated cells have all been studied, but skeletal muscle has received the most attention. This is true partly because the motion of muscle cells can be stopped and started at the will of the experimenter. This fortunate property, absent in amoeboid and ciliated cells, allows the muscle cell to be compared with itself during rest and exercise. Metabolic poisons can be used to stop movement in non-muscular cells, but chemical inhibition is seldom reversible or specific enough for experimental designs as elegant as those possible in studies on muscle.

Recently, genetic mechanisms have been discovered for controlling the motility of certain flagellated cells: the bacterium *Salmonella typhimurium* (Stocker, Zinder and Lederberg, 1953) and the autotrophic green alga, *Chlamydomonas* (Lewin, 1952). Of the two organisms, *Chlamydomonas* has some advantages as an experimental object, since it is nonpathogenic and has simple, well-defined nutrient requirements. By using ultraviolet light, Lewin (1954) has produced several single-locus mutant strains with abnormal flagellar characters, including some which look just like the wild-type strain but do not move their flagella. The paralysis must be related to an abnormality either of flagellar structure or of some other part of the cell. The failure of Mintz and Lewin (1954) to find serological differences between the flagella of normal and paralyzed strains suggests that these flagella may be structurally similar. If this is so, the loss of motility is probably related to a metabolic change elsewhere in the cell. It is now possible by using these algal strains to compare the metabolism of "normal" and "paralyzed" flagellated cells which are presumably alike in other respects. For this comparison it is necessary to assume that a large and definite proportion of the cells in the "normal" culture is motile. An estimate of this proportion, the motility index, will be developed primarily for use in later studies. Its use in this paper will be only to justify the above assumption.

The energetic cost of flagellar motion will be estimated in two ways. One estimate is based on microscopic study of the motile cells, the other on measurements of respiration. The two estimates will be compared.

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Methods

The marine organisms used in this study, *Amphidinium Klebsi, Carteria* (?) sp. and *Dunaliella* sp., were obtained from Dr. J. H. Ryther at the Woods Hole Oceanographic Institute. The three strains of *Chlamydomonas* came from the Department of Botany, Indiana University (I.U.): they were *C. Moewusii* (+) (I.U. No. 97) herein called "CMW," *C. Moewusii* (+) (Lewin's paralyzed strain No. M 1001; I.U. No. 697) herein called "CMP," and *C. Reinhardi* (+) (I.U. No. 89, Sager and Granick, 1953) herein called "CRW." Marine organisms were studied in filtered, autoclaved Woods Hole sea water and kept on agar slants made with sea water. Fresh-water *Chlamydomonas* was grown and studied in a liquid medium suggested by Fuller [2] which contained

KNO_3, 1 *M*	5.0 ml.
K_2HPO_4, 1 *M*	0.5 ml.
KH_2PO_4, 1 *M*	0.5 ml.
$MgSO_4$, 1 *M*	2.0 ml.
$Ca(NO_3)_2$, 1 *M*	0.25 ml.
"Trace element solution"	1.0 ml.
"Iron solution"	1.0 ml.
Iron-free water to make	1000 ml.

"Trace element solution" contained

H_3BO_3	1.43 g.
$MnSO_4 \cdot H_2O$	1.05 g.
$ZnCl_2$	0.05 g.
$CuSO_4 \cdot 5H_2O$	0.04 g.
$H_2MoO_4 \cdot H_2O$	0.01 g.
Distilled water to make	1000 ml.

"Iron solution" contained

Disodium ethylenediaminetetra-acetate	0.5 g.
$FeSO_4 \cdot 7H_2O$	5.0 g.
Distilled water to make	1000 ml.

The culture vessels were 125-ml. Erlenmeyer flasks containing 50 ml. of medium and 2.5-liter, wide, flat-bottomed culture flasks (like A. H. Thomas No. 4372-F) containing one liter of medium. Air containing 5% CO_2 was bubbled through the larger cultures. The flasks were shaken mechanically to swirl the contents gently. Four fluorescent lamps (type F40T12W or SW) were mounted under a glass-bottomed water thermostat kept at 23° C., in which the larger flasks were immersed to the level of the medium inside. The illumination 2.5 cm. above the bottom of the flasks was about 500 foot-candles as estimated with a photographic exposure meter. The large flasks were inoculated either with 100 ml. of a previous one-liter culture, or with a 50-ml. culture, reared for the purpose in a

[2] R. C. Fuller, personal communication (1955).

small flask. The small flasks were illuminated from above and shaken gently but were not otherwise aerated. One-liter cultures were ready for harvest (about 2×10^6 cells ml.$^{-1}$) in two to four days, depending on the inoculum. The harvest was usually concentrated by gentle centrifugation, and the cells were re-suspended in fresh medium before use.

Motility of whole populations of cells was studied by comparing photomicrographs of samples of cell suspensions. The film (Du Pont Microcopy) was exposed for 8 seconds and developed for maximal contrast with elon-hydroquinone contrast developer (Kodak formula No. D-11). After being processed, the photographs were projected onto a screen for counting those cells which were stationary long enough to form images. Images of moving cells failed to register because of the long photographic exposure. The use of a haemacytometer [3] and a phase-contrast microscope in photography made the counting easier.

This method leads easily to the formulation of a *motility index, M*. A practical definition of M is

$$M = 1 - \frac{n_1 - n_2}{n_3},$$

in which n_1 = the number of cells counted in a defined area of the photograph of a cell suspension, made with a time exposure of 8 seconds;

n_2 = the number of cells in the first photograph whose images fail to appear in the second, otherwise similar, photograph taken one minute later;

n_3 = the number of cells appearing in a photograph of a different drop of the same suspension, in which all the cells are immobilized (*e.g.*, with HCHO or I_2 vapor).

The second measure of motility used here is based on the speed of locomotion of individual motile cells in a drop of a dilute suspension, placed on a slide and covered with a coverglass, at room temperature (21 to 23° C.). The individuals to be studied were selected at random by tracking every cell which crossed a line bisecting the field, for as long as it remained in the field. The image of the cell was projected on to a sheet of paper, using a camera lucida. The path of motion was described by pencil marks indicating the position of the cell every two seconds. A loudly ticking clock or mechanical sounder was found to be essential. The distance travelled by the cell per second was calculated from a summation of the line segments connecting the pencil marks on the sheet, and from the time elapsed between the placement of the first and the last marks. The distances travelled per second by several cells in the same suspension were averaged to estimate the *average speed of locomotion* for the population.

Oxygen consumption was measured at 23° C. by the Warburg method. Each 14-ml. reaction vessel was inclosed in a light-tight cloth bag and contained 2 ml. of a suspension of cells which had been washed by gentle centrifugation ($700 \times G$, 30 seconds) and re-suspended in fresh medium. The manometers were read every 10 minutes. The respiratory rate was found to decline slowly with time, but

[3] A haemacytometer chamber for phase-contrast microscopy is manufactured by the American Optical Co.

not appreciably during the first 90 minutes; the readings during this period were fitted with a straight line by the method of least squares. Respiratory rates were then expressed in μl. of O_2 (S.T.P.) per mg. total nitrogen per hour (Q_{O_2} (N)). Total N was estimated by sulfuric-acid digestion of an aliquot, with three successive additions of H_2O_2, followed by direct Nesslerization and reading of the samples in a Klett-Summerson photoelectric colorimeter (Miller and Miller, 1948).

Results

Degrees of motility in a culture of C. Reinhardi

When samples of a culture of *C. Reinhardi* were observed with the microscope, they were found to contain some stationary cells. Some of these became motile from time to time; at the same time swimming individuals settled down to become members of the stationary group. In general the stationary group seemed to remain constant in size; therefore, in any series of observations the number of *originally* stationary cells becoming active in any time interval may be expected to bear a constant relation to the number of *originally* stationary cells remaining. To test this supposition, a single drop of a culture was photographed repeatedly over a period of several minutes. The photographs were studied, and numbers of originally stationary cells remaining were plotted on a logarithmic scale against time. In one experiment (Fig. 1) the points fell on a straight line for the first

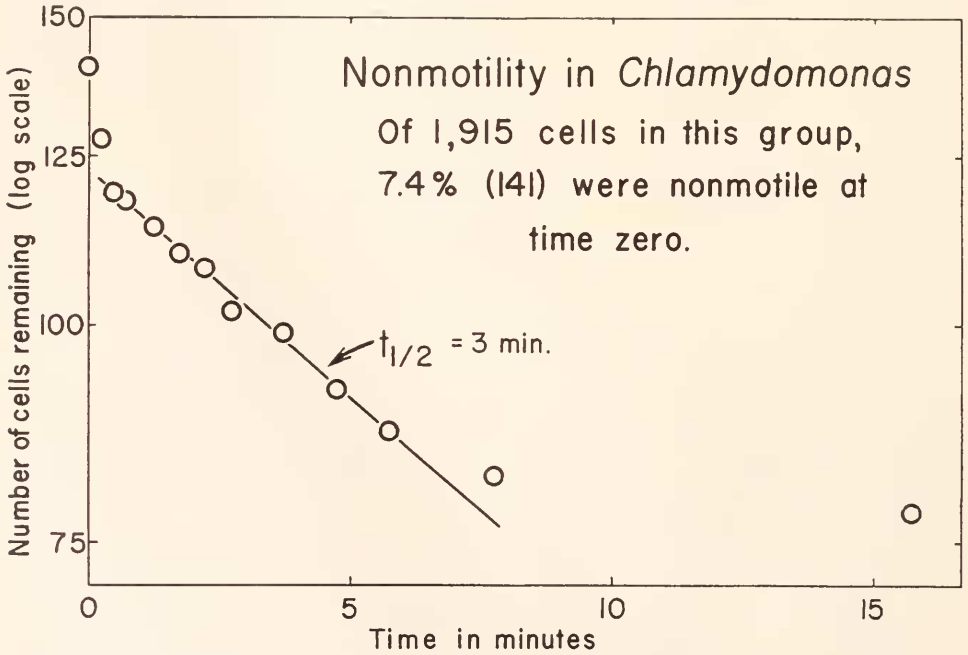

Figure 1. Degrees of motility in a culture of *Chlamydomonas Reinhardi*. For the first 5 minutes many of the originally stationary cells became motile as shown by the points fitted with a straight line. During the first 30 seconds a more active group of cells dominated; a slower, possibly more heterogeneous group dominated after 5 minutes.

five minutes; this supports the hypothesis that a constant proportion of the remaining non-motile cells become active during each time interval. However, the graph also revealed that the entire original population of stationary cells was made up of three classes, according to their rates of decrease. The first two had half-times of one and three minutes, respectively. Cells in the third class, possibly including dead individuals in the culture, failed to move in 16 minutes. For this population the first photograph showed 141 (n_1) stationary cells; in the second photo 23 (n_2) of these particular cells were missing. A photo of a killed sample showed 1915 (n_3) cells. Thus, $M = 0.94$. In general, samples from other cultures gave similar results, except that the "one-minute" class often could not be found.

Locomotion of individual cells of several species

A different quantitative concept of cellular motion results from the detailed observation of single motile cells selected at random from a culture. The path of motion of a flagellated algal cell is a series of straight lines or arcs of large radius. Cells may occasionally change direction abruptly or spin briefly in place as if held by a mucous attachment. In addition, cells which are swimming forward often revolve about an axis parallel to the direction of motion (Brown, 1945) and may oscillate as they swim.

For studies of the velocity of motion, several kinds of elliptical or nearly spherical flagellates were selected. Table I shows the observations and calculations derived from them. The "average radius" is one fourth the sum of length and width. The minimal power dissipation, P, per cell was calculated from Stokes's Law relating to the force, f, needed to propel a sphere through a fluid:

$$f = 6\pi r\eta u,$$

and from the relation

$$P = 10^{17} fu,$$

where

f = force needed to overcome fluid resistance (dyne),
r = average radius of cell (cm.),
η = viscosity of fluid (poise = dyne sec. cm.$^{-2}$),
u = average speed of locomotion (cm. sec.$^{-1}$),
P = power (watt = 10^7 dyne cm. sec.$^{-1}$).

Oxygen consumed by normal and by paralyzed Chlamydomonas

A third aspect of flagellar motion concerns the intensity of metabolism of the flagellated cell. In this study, the oxygen consumption of a population of normal *Chlamydomonas Moewusii* (CMW) was compared with that of the ultraviolet-induced, "paralyzed" mutant (CMP). The mutant cells have flagella but fail to use them; these are held out rigidly almost perpendicular to the main axis of the cell. Occasionally a flagellum showed a little motion at its tip, but this hardly ever caused the whole cell to move.

The figures in Table II are based on 12 reaction vessels for CMW and 13 for CMP. In preparation for each experiment the cells of the two strains were reared

TABLE I

Minimal power output of selected flagellated cells

Species	Size		Medium	Aver. velocity 10^{-2} cm. sec.$^{-1}$	Min. power output/cell, 10^{-16} watt
	Aver. radius 10^{-4} cm.	Length / Width			
Amphidinium Klebsi	7.98 (11)	1.30	Sea water	0.739 (11)	7.5
Carteria (?) sp.	6.54 (9)	1.67	Sea water	1.25 (10)	17
Chlamydomonas Moewusii (CMW)	5.54 (11)	1.48	Fresh water	1.28 (16)	15
Dunaliella sp.	4.40 (9)	1.35	Sea water	2.26 (9)	38
Chlam. Reinhardi (CRW)	3.26 (100)	1.08	Fresh water	0.828 (100)	3.9

Numbers of individuals studied are in parentheses. Viscosities (corrected for density) used in calculations were: sea water, 0.965 cp (estimated from Miyake and Koizumi, 1948); fresh water, 0.931 cp.

in one-liter cultures under identical conditions. In each experiment the oxygen consumption of the paralyzed cells was less than that of the normal cells when expressed in terms of total cellular nitrogen.

DISCUSSION

The quantitative description of cellular motility will be discussed before considering the energy required for flagellar motion. This study presents two quantitative methods of studying locomotion in populations of flagellated cells.

The *motility index* (photographic method) can be used for distinguishing the behavior of cell populations exposed to varying experimental treatments. It may prove helpful in pharmacologic and toxicologic studies on suspensions of algae, protozoa, bacteria, or sperm cells; these forms may offer the experimenter advantages over larger and more expensive animal subjects. Compared with other proposed estimates of the proportion of non-motile cells in a microscopic field (Emmens, 1947; White, 1954) it would appear to avoid certain subjective errors in sampling and counting, and to minimize the error due to the inclusion of cells

TABLE II

Oxygen consumption (Q_{O_2} (*N*)) *of normal and paralyzed Chlamydomonas Moewusii (paired comparison)*

Experiment no.	CMW	CMP	Difference (CMW-CMP)
6-27-57	1.24	1.14	0.10
7- 1-57	1.36	1.24	0.12
7-22-57	1.31	0.79	0.52
8- 3-57	1.19	1.14	0.05
8- 5-57	2.33	2.06	0.27
8- 7-57	2.00	1.72	0.28
Mean difference and its standard error ($n = 6$)			0.223 ± 0.0706

which may stop for momentary "rest" periods. It cannot distinguish degrees of impairment of locomotion.

The *average speed of locomotion* appears to be valuable for distinguishing populations of cells which show normal speeds of locomotion from populations with impaired locomotion. It takes no notice of non-motile cells, and thus becomes most useful in estimating the degree of motility in cultures where the motility index is high. It is similar in principle to one devised by Baker, Cragle, Salisbury and Van Demark (1957) who measured the time required for 100 free-swimming sperm cells to pass through a segment of a plane. Their method, which seems admirably suited to cells displaying the sperm type of locomotion, has the advantage of presenting the result of an experiment immediately without waiting for photographic processing. The decision to use a given method will rest partly upon the extent to which its assumptions are fulfilled by the swimming habits of the organism. The method described here is of special value, since from it can be derived an estimate of the external work done by the motile cells in the population.

The estimates of power dissipation in Table I are certainly low, because the premises on which they are based all tend to reduce the estimates. It is supposed, for example, that the cell's internal energetic conversions are 100% efficient. The other assumptions, each known to be false to some extent, are: that there are no degrees of motion other than uniform in a straight line (contradicted by Brown, 1945, and others), that the cell is a sphere (contradicted in Table III), and that

TABLE III

Estimates of size of Chlamydomonas

Strain.....	CRW	CMW	CMP
Length, μ	6.49 ± 0.14	7.64 ± 0.17	7.41 ± 0.14
Width, μ	5.24 ± 0.17	5.62 ± 0.14	4.92 ± 0.10

The "±" sign is inserted between the mean and its standard error. Fifty cells of a single culture of each strain were measured.

the frictional drag of the flagellum, apparently of major importance in the locomotion of sea urchin sperm (Gray and Hancock, 1955), is negligible in *Chlamydomonas*. Excepting *C. Reinhardi,* the smaller flagellates travelled faster and displayed a higher power output than the larger cells. Whether this difference is related to a greater metabolic rate of the smaller cells has not been determined.

When normal and paralyzed *C. Moewusii* were reared and studied under the same conditions in several successive experiments, the paralyzed cells (CMP) always consumed less oxygen than did the wild-type, motile cells (CMW). The average difference in Q_{O_2} (N) was about 14% of that of the normal cells, and was found to be statistically significant (t-test, $n = 6$) at the 5% level, but not at the 2% level. It must be assumed that the proportion of dead cells in the CMP culture is no greater than in the CMW culture. In interpreting this difference, certain other features of the two strains should be borne in mind.

Ocular micrometer measurements showed that although CMW and CMP are of equal length, the paralyzed cells are, on the average, a little more slender than

the motile ones (Table III). Thus, a paralyzed cell's surface-to-volume ratio is slightly greater than that of a normal cell. From the size of this difference alone one would expect the Q_{O_2} (N) of the paralyzed strain to be a little greater than that of the normal strain; it appears in fact to be less. The single mutation which resulted in paralysis of the flagella may have had other expressions, possibly involving alterations in the efficiency of biochemical pathways of metabolism. In summary, the physiologic differences between the two strains may be much greater than appeared at first. In ignorance of the magnitudes of these possible factors, it is tempting to suggest that the difference in oxygen consumption is actually related to the state of motility of the cell, but a cautious attitude seems desirable.

As a partial test of this relationship, we may now compare the two available estimates of the energy required for motility. One of these (Table I) states that *C. Moewusii* dissipates at least 10^{-15} watt per cell in overcoming the frictional losses in water. The other estimate is derived from the difference in Q_{O_2} (N) between the normal and paralyzed strains, which is 0.22 μl. hr.$^{-1}$ (mg. N)$^{-1}$. If we suppose that the exclusion of light from the Warburg vessel does not affect motility (Lewin, 1953), the two figures are comparable; the latter figure can then be transformed to watts per cell by making the following reasonable assumptions:

1. The consumption of 1 μl. of O_2 releases about 4.8×10^{-3} calorie or 5.58×10^{-6} watt hour.
2. A CMW cell contains 2.65×10^{-9} mg. N (estimated from cell counts and N determinations on a single culture at the time of harvest).
3. The motility index in the CMW culture is high.

The observed difference in the rate of oxygen consumption thus corresponds to a difference in power dissipation of 3.3×10^{-15} watt per cell. Rothschild's (1953) reworking of Taylor's figures gives estimates close to these for the minimal energy dissipated by bull sperm: for two kinds of assumptions, 3.74×10^{-14} watt and 2.04×10^{-15} watt per cell. In our comparison, the efficiency of conversion of chemical to mechanical energy is not taken into account. The closeness of the two estimates derived in this paper suggests that the lower rate of oxygen consumption of paralyzed cells may be correlated with their loss of motility.

Summary

1. The paper describes a method for estimating the minimal power output of individual, nearly spherical, flagellated cells. A comparison of 5 species of green flagellates suggests no relationship between size and power dissipation (Table I).

2. A simple photographic method for estimating the fraction of motile organisms in a culture is described.

3. Cultured populations of *Chlamydomonas Reinhardi* may contain two or more distinct groups of cells with different degrees of motility (Fig. 1).

4. The motile, "wild-type" *C. Moewusii* consumed 1.57 μl. O_2 (S.T.P.) per hour per mg. total N.

5. A paralyzed mutant strain of the same species consumed 14% less oxygen than the wild type. The extra oxygen consumed by the motile strain is commensurate with its estimated minimal power output.

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